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# Compatibility of the biocontrol fungus *Fusarium oxysporum* strain CS-20 with selected fungicides

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**Abstract**

*Fusarium oxysporum* strain CS-20 reduces incidence of Fusarium wilt in tomato and other crops. To be integrated into most production systems, strain CS-20 must be compatible with other management practices. We assessed the compatibility of strain CS-20 with seven fungicides recommended for tomato in Maryland. Radial growth of strain CS-20 was recorded on agar medium amended with various concentrations of the fungicides. Fungicides tested did not kill strain CS-20 at the concentrations tested in the in vitro experiment. Azoxystrobin (Quadris) and chlorothalonil (Bravo) were most toxic to strain CS-20 and significantly reduced growth rate and final colony size at 10 ppm a.i. or greater concentrations compared to growth on unamended medium. Thiram (thiram) significantly reduced final colony size at 30 ppm or greater. Mefenoxam + chlorothalonil (Ridomil Gold Bravo) significantly reduced final colony size at 50 ppm or greater. Mancozeb (Manzate) and mancozeb + copper (Mankocide) reduced final colony size only at 100 ppm, while mefenoxam (Ridomil Gold) and mefenoxam + copper (Ridomil Gold Copper) did not affect growth of strain CS-20. In greenhouse tests, tomatoes were drenched with strain CS-20 at seeding and just before transplanting into field soil infested with the pathogen. Plants were treated with fungicides at the highest label rate. Mancozeb is labeled as both a seed treatment and a spray, and was applied each of these ways as separate treatments. Disease incidence of plants from seeds treated with thiram and strain CS-20 was not different from those in the pathogen only control, indicating that thiram was toxic to strain CS-20. Other fungicides toxic in vitro, were less toxic in greenhouse tests, probably because they are applied as sprays to the above-ground portions of the plant. Published by Elsevier Inc.

*Keywords:* *Fusarium oxysporum*; Fusarium wilt; Biopesticide; Azoxystrobin; Chlorothalonil; Mancozeb; Mefenoxam; Thiram

## 1. Introduction

Development of alternative control strategies such as biocontrol can provide additional management tools as supplements to other control measures for plant diseases, for use in rotation with other control measures, or as back-up when favored control measures are withdrawn from the market or fail due to new strains or races of the pathogen.

Fusarium wilt, caused by *F. oxysporum* f. sp. *lycopersici*, is a serious problem for tomato production in many areas. The disease is managed through resistant cultivars and pre-plant soil fumigation. Because new races of the pathogen can develop and fumigants are being lost from the market, alternative control strategies are being investigated. There are many examples in the literature of using nonpathogenic *Fusarium* spp. to control Fusarium wilts (reviewed in Fravel et al., 2003). In field and greenhouse studies, *F. oxysporum* strain CS-20 has demonstrated potential for reducing the incidence of Fusarium wilt on tomato, muskmelon and basil (Larkin and Fravel, 1998, 1999a,b, 2002a,b; Larkin et al., 1999).

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To successfully integrate biocontrol organisms into production systems, the compatibility of these organisms with pesticides used in the production system must be known. This work was undertaken to determine the compatibility of *F. oxysporum* strain CS-20 with fungicides recommended for use on tomato in Maryland.

## 2. Materials and methods

### 2.1. In vitro experiments

The biocontrol strain *F. oxysporum* strain CS-20 was originally isolated in Florida from a soil suppressive to Fusarium wilt of watermelon (Larkin et al., 1996). For in vitro experiments, the fungus was grown on potato dextrose agar (PDA; Sigma, St. Louis, MO) at 25 °C for 1 week. One-half centimeter disks from the growing edge of the fungal colony were transferred to the center of fresh PDA amended to result in 0, 10, 30, 50, or 100 ppm a.i. of each of the following fungicides: azoxystrobin (Quadris; Syngenta, Greensboro, NC); chlorothalonil (Bravo Ultrex; Syngenta); mancozeb (Manzate 75DF; Griffin, Valdosta, GA); mancozeb + copper (Mankocide DF; Griffin); mefenoxam (Ridomil Gold EC; Syngenta); mefenoxam + chlorothalonil (Ridomil Gold Bravo, Syngenta); mefenoxam + copper (Ridomil Gold Copper, Syngenta); and thiram (Thiram 50WP Dyed; Gustafson, Plano, TX) (Table 1). Active ingredient (a.i.) refers to the sum of the two active ingredients when two are present in the fungicide as it is sold. Agar was amended by preparing 100 ml of PDA and adding 1 ml of sterile distilled water (SDW) or the appropriate fungicide dilution brought up to 1 ml with SDW in sterile Eppendorf tubes. Eighteen milliliters of medium was dispensed into each of four replicate 9-cm diameter plastic petri plates. Two perpendicular lines were drawn on the bottom of each petri plate and the fungal disk was placed at the intersection of the two lines.

The diameter of the fungus was measured on each of the two perpendicular lines drawn on the plate every 2–3 days until the fungus reached the edge of the plate (up to 14 days). Data were analyzed by a general linear model (proc glm, SAS, Cary, NC). Growth rate was calculated. The experiment was repeated once.

### 2.2. Greenhouse experiments

Inoculum of strain CS-20 and the pathogen, *F. oxysporum* f. sp. *lycopersici* race 1, were grown in an aqueous suspension of 1%(w:v) soy hull at 25 °C with shaking (100 rpm) for 2–4 weeks before use (Hebbar et al., 1996). Spores (microconidia + macroconidia + chlamydospores) were enumerated with a hemacytometer and spore concentrations were adjusted with SDW.

For fungicides labeled for seed use (manzate, thiram), tomato seeds (*Lycopersicon esculentum* cv. Bonny Best) were treated as described on the product label. Treated or nontreated seed were planted in soilless potting mix (Redi-Earth; Scotts-Sierra, Marysville, OH). Five seeds were planted in each cell of plastic seedling trays (98 cells, $3.3 \times 3.3 \times 5$ cm tall). One cell with five seeds was considered a replicate. Each replicate was drenched with 5 ml of a $10^6$ spores/ml aqueous suspension of strain CS-20. After 6 weeks, each replicate was again drenched with 5 ml of a $10^6$ spores/ml aqueous suspension of strain CS-20. The following day, each replicate (4–5 plants) was transplanted into a 15 cm diameter plastic pot with nonsterile field soil (Galeston gravely sandy loam) infested with $10^4$ spores/g soil of the pathogen. Treatments were replicated 10 times. Plants were sprayed at 1, 3, and 5 weeks after transplant with the maximum label rate of azoxystrobin, chlorothalonil,

Table 1
Label rates, chemical components, chemical class, and mode of action of fungicides used in experiments

| Fungicide | Highest label rate | Component | Chemical class | Mode of action |
|---|---|---|---|---|
| Bravo Ultrex | 2.40 kg a.i./ha | Chlorothalonil | Substituted benzene chloronitrile | Combines with $NH_2$ or SH group of essential metabolic compounds |
| Manzate 75DF | 2.52 kg a.i./ha | Mancozeb | Ethylene bisdithiocarbamate | Inactivates SH groups in amino acids |
| Mankocide DF | 3.42 kg a.i./ha | Mancozeb | Ethylene bisdithiocarbamate | Inactivates SH groups in amino acids |
| | | Copper hydroxide | Copper | Nonspecific denaturization of protein |
| Ridomil Gold Bravo | 1.46 kg a.i./ha | Mefenoxam | Phenalymide | Depresses nucleic acid synthesis |
| | | Chlorothalonil | Substituted benzene | Combines with $NH_2$ or |
| | | | Substituted aromatic | SH group of essential metabolic compounds |
| Ridomil Gold Copper | 1.46 kg a.i./ha | Mefenoxam | Phenalymide | Depresses nucleic acid synthesis |
| | | Copper | Copper | Nonspecific denaturization of protein |
| Ridomil Gold EC | 1.17 L a.i./ha | Mefenoxam | Phenalymide | Depresses nucleic acid synthesis |
| Thiram 50WP Dyed | 1.87 g a.i./kg seed | Thiram | Dithiocarbamate | Interferes with oxygen uptake and inhibition of sulfur containing enzymes |
| Quadris | 103.76 ml a.i./ha; 112.09 g a.i./ha | Azoxystrobin | Pyridimine | Inhibits biosynthesis; sterols |

mancozeb, mancozeb + copper, mefenoxam + copper, or mefenoxam + chlorothalonil (Table 1).

At 6 weeks after transplant, the basal potion of each stem was collected, surface disinfested for 1 min in 10% sodium hypochlorite, rinsed in SDW, and plated onto a medium semi-selective for Fusarium (Komada, 1975). Recovery of Fusarium was recorded. Data for each replicate consisted of the percentage of stems from which Fusarium was recovered and this was considered disease incidence for that replicate. Data were analyzed by analysis of variance and a Duncan's multiple range test (SAS). Normality of the data was confirmed by performing a Kruskal–Wallis test (SAS). The experiment was repeated once.

## 3. Results

### 3.1. In vitro experiments

Fungicides tested did not kill strain CS-20 at the concentrations tested in the in vitro experiment. Azoxystrobin and chlorothalonil were most toxic to strain CS-20 and significantly reduced growth rate and final colony size at 10 ppm or greater concentrations compared to growth on unamended PDA ($P \leqslant 0.05$; Fig. 1). Thiram significantly reduced final colony size at 30 ppm or greater. Mefenoxam + chlorothalonil significantly reduced final colony size at 50 ppm or greater. Mancozeb and mancozeb + copper reduced final colony size only at

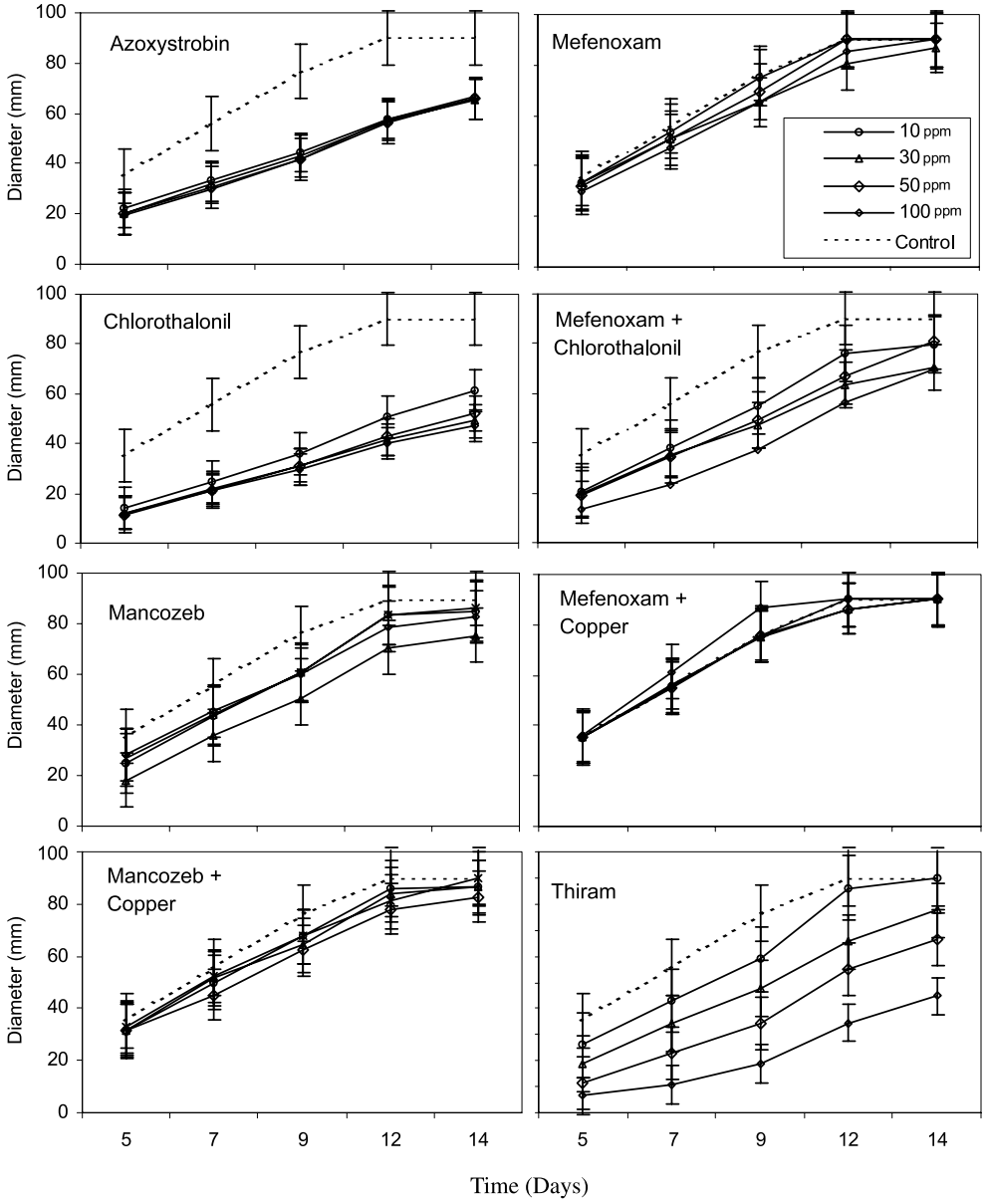


Fig. 1. Effect of four concentrations of fungicides incorporated into potato dextrose agar on radial growth of the biocontrol fungus *F. oxysporum* strain CS-20. Bar represent standard error of the mean.

100 ppm, while mefenoxam and mefenoxam + copper did not affect growth of strain CS-20.

### 3.2. Greenhouse experiments

Compared to the pathogen alone control treatment (72.3%), strain CS-20 significantly reduced the incidence of recovery of Fusarium from surface-disinfested stems (23.3%), indicating biocontrol by strain CS-20 (Table 1). All fungicides tested appeared to reduce the effectiveness of strain CS-20 to some degree, although only thiram and mefenoxam + chlorothalonil significantly impaired the ability of strain CS-20 to reduce disease incidence. Mefenoxam as a seed treatment was included in only one run of the experiment and thus is not shown in the combined analysis presented here. When it was included, the response in the mefenoxam treatment was intermediate between mefenoxam + copper and mancozeb (see Table 2).

## 4. Discussion

Knowledge of compatibility of biocontrol agents with other components of the production system is needed to develop feasible management strategies. Yeasts used as biocontrols for postharvest diseases have been studied for compatibility with pesticides and adjuvants. The biofungicide AQ10 (*Ampelomyces quisqualis*) was compatible with the chemical pesticide triadimefon and the adjuvant AddQ (Shishkoff and McGrath, 2002). In a trial in a commercial packinghouse, combining the yeast *Cryptococcus laurentii* plus half the label rate of thiabendazole was significantly more effective than the label rate of the fungicide whenever thiabendazole-resistant spores of the pathogen were present for control of blue mold of pear (Chand-Goyal and Spotts, 1997). In another packinghouse test, Aspire (*Candida oleophila*) plus 100 ppm thiabendazole controlled decay of pear as well as 569 ppm of thiabendazole, the maximum label rate (Sugar and Spotts, 1999). Other biocontrol agents have also been tested with reduced rates of fungicides. Disease incited by *Rhizoctonia solani* was reduced on rosemary by combining *Laetisaria arvalis* and a foliar spray of half the rate of an experimental fungicide (Conway et al., 1997).

Lack of knowledge of compatibility of biocontrol agents with pesticides may contribute to failure of biocontrol to perform as expected. It is possible that the lack of control of Fusarium wilt of muskmelon in one field trial was in part due to the use of seed treated with fungicide (Bao et al., 1999).

In interpreting data from the current study, characteristics of strain CS-20 should be kept in mind. Strain CS-20 provides biocontrol of Fusarium wilt primarily through a host-mediated mechanism (Larkin and Fravel, 1999b). Strain CS-20 superficially colonizes the interior of tomato roots, but does not penetrate the vascular system (Fravel, unpublished). In all our studies, CS-20 has been applied as two separate soil drenches, one at seeding and the second a day prior to transplant, but it is not know whether both drenches are needed. In the present study, two fungicides are on the seed before the first drench (mancozeb and thiram). The other fungicides are all applied to the foliage beginning 1 day after the second application of strain CS-20.

In our study, azoxystrobin and chlorothalonil were the most toxic fungicides to strain CS-20 in vitro, but were least toxic in the greenhouse tests. This is likely due to the fact that CS-20 and the fungicides were spatially and temporally separated in the greenhouse tests. CS-20 was applied as a drench at seeding and just prior to transplant. Thiram was toxic to CS-20 in vitro, and because it is used as a seed treatment, it greatly impaired the ability of strain CS-20 to reduce pathogen infection. Thus, it is important that compatibility be assessed in a way as similar as possible to how the biocontrol agent and fungicide will be used in the production system.

Table 2
Incidence of recovery of Fusarium (disease incidence) from surface-disinfested tomato treated with or without the biocontrol fungus *F. oxysporum* strain CS-20 plus various fungicides[a]

| Treatment | Fusarium recovery (%)[b] |
|---|---|
| Pathogen only | 72.3 a |
| Thiram (seed) with CS-20 | 50.2 b |
| Mefenoxam + chlorothalonil with CS-20 | 43.2 bc |
| Mefenoxam + copper with CS-20 | 37.3 bcd |
| Mancozeb (seed) with CS-20 | 36.1 bcd |
| Mancozeb with CS-20 | 33.2 cd |
| Mancozeb + copper with CS-20 | 32.5 cd |
| Azoxystrobin with CS-20 | 27.3 d |
| Chlorothalonil with CS-20 | 27.1 d |
| Pathogen with CS-20 | 23.3 d |
| CS-20 only | 8.2 e |
| Water only | 1.0 e |

[a] Except as noted where applied to seed, fungicides were sprayed weekly at the highest label rate beginning 1 week after transplant. All treatments except water only and strain CS-20 only are in pathogen-infested soil.

[b] Values followed by the same letter are not significantly different from each other according to Duncan's multiple range test ($P \leqslant 0.05$). Data presented are the combined data from the two runs of the experiment.

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